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Survival of microbes in Earth's stratosphere

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The remarkable survival of microorganisms high above the surface of the Earth is of increasing interest. At stratospheric levels, multiple stressors including ultraviolet and ionizing radiation, low temperatures, hypobaric conditions, extreme desiccation, and nutrient scarcity are all significant challenges. Our understanding of which microorganisms are capable of tolerating such stressful conditions has been addressed by stratospheric sample collection and survival assays, through launching and recovery, and exposure to simulated conditions in the laboratory. Here, we review stratospheric microbiology studies providing our current perspective on microbial life at extremely high altitudes and discuss implications for health and agriculture, climate change, planetary protection, and astrobiology.

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Introduction

While the atmosphere represents the largest fraction of the biosphere by volume, it contains by far the lowest number of cells. Estimates have suggested that the atmosphere, primarily the troposphere (<5 to 18 km above sea level), contains less than a billionth the number of cells found in the oceans, soils, and subsurface [1]. At stratospheric elevations, from 12–18 km to 50 km above sea level, the number of cells declines and temperature, oxygen, and humidity levels all plummet (Figure 1). Above the ozone layer (~15 to 35 km), ultraviolet (UV) and cosmic radiation quickly become lethal factors (Box 1). At even higher elevations, above 50 km, into the mesosphere and thermosphere, life as we know it may be impossible.

Microorganisms of terrestrial origin enter the stratosphere by vertical movement of air from the troposphere as a result of thunderstorms, dust storms, volcanic action, and human activity (Box 2). Over 10^{21} cells are lifted annually into the atmosphere, leading to considerable transport and dispersal around the globe, even with a very small fraction (<0.1%) surviving due to the extreme conditions [1,2,3•]. Exact cell counts in the stratosphere are yet to be fully established, but some sporulating and non-sporulating bacteria and fungi are regularly recovered (Table 1). Moreover, certain widely distributed species, such as extremophilic archaea and pathogenic bacteria, have been shown to survive residency in the stratosphere, at least for short periods of time [4••,5••].

Microbes isolated from the stratosphere

Early sampling expeditions revealed that the upper limit of terrestrial life is at a very high altitude, above the troposphere, in the stratosphere, or possibly even higher [6]. In 1936, Rogers and Meyer reported the first successful air sampling mission reaching the stratosphere (11–21 km). Using balloons with autoclaved collection tubes, they isolated both viable bacteria and fungi, including *Bacillus* and *Aspergillus* spp. (Table 1). In 1965, Soffen reported isolating only *Penicillium* sp. using an impactor which had been sterilized by ethylene oxide for air sampling at 40 km also using balloons. In the 1970s, Imshenetsky and co-workers sampled air from 48 to 85 km elevation using meteorological rockets sterilized by γ-radiation. Remarkably, isolates were reported up to 77 km, and primarily the same types of fungi as previously reported in stratospheric sampling. In addition, the non-spore forming *Micrococcus albus* and *Mycobacterium luteum* bacteria were also found (Table 1).

In more recent sampling expeditions, enhanced technology for sterilization of sampling devices, and culturing and non-culturing methods have been refined [7•,8–10,11••]. A balloon-borne cryosampler was used to collect material from 41 km, resulting in isolation of viable *Bacillus simplex*, *Staphylococcus pasteuri* and the fungus *Engyodontium albus* [12]. An impactor device mounted on the underside of a high altitude research aircraft at 20 km was used to collect spore-forming *Penicillium* sp., *Bacillus luciferensis* and *B. sphaericus* [13]. In a subsequent mission, non-spore-forming bacteria from the families *Micrococcaceae* and *Microbacteriaceae*, and genera *Staphylococcus* and *Brevibacterium* were isolated [14]. Interestingly, most *Micrococcaceae* and several *Microbacteriaceae* matched to previously identified strains from volcanic soils, suggesting that these may be lofted into the stratosphere during eruptions. Delayed growth and smaller colony

Figure 1

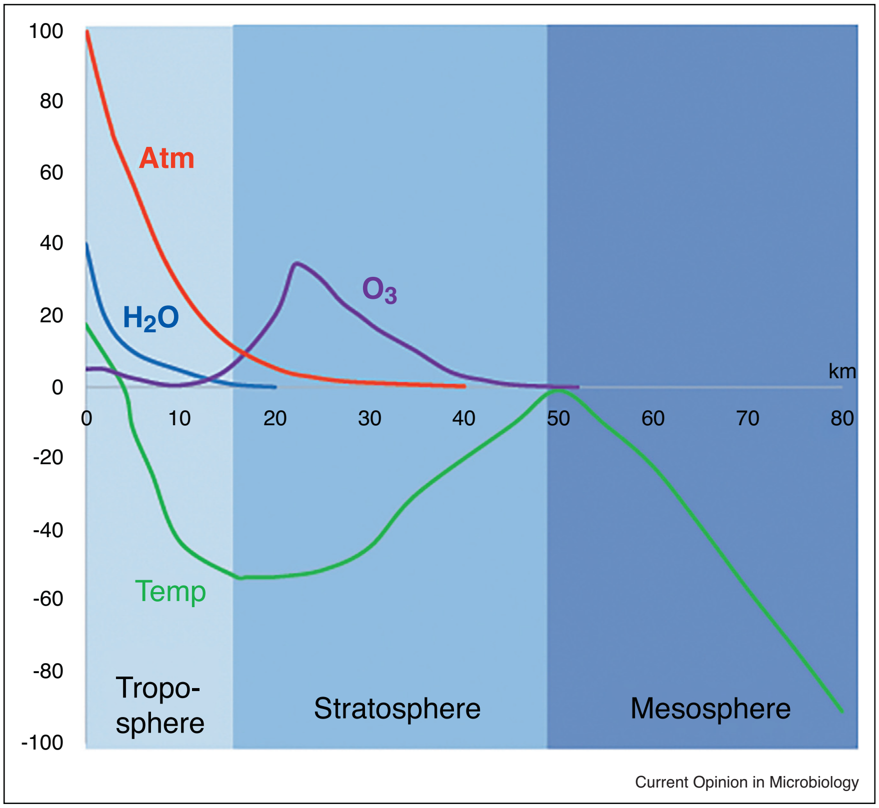

Diagram displaying characteristics of the atmosphere. Atmospheric temperature (Temp, in °C, green), atmospheric pressure (Atm, in kPa, red), water vapor (H_2O, in g/m^3, blue), and ozone (O_3, in mPa, purple) on the *Y*-axis are all plotted against elevation above sea level (ASL in km) on the *X*-axis.

Box 1 Major stratospheric stressors

- UV-C radiation
- Cosmic radiation
- Freezing temperatures
- Hypobaric pressure
- Desiccating condition
- Starvation
- Ozone

Box 2 Activities affecting the stratosphere

Human	Nature
• Commercial jet aircraft	• Volcanic eruptions
• Meteorological balloons	• Tropical storms
• Military aircraft and rockets	• Thunderstorms
• Spacecraft and satellite transit	• Dust storms

formation were noted for the isolates and attributed to cell stress [14].

In a mission to 11–12 km elevation, slow-growing microbes in the *Deinococcus* genus were isolated using an airdust sampler, and later classified as *Deinococcus aetherius*. Several *Bacillus* spp. and a *Paenibacillus* strain were also identified from the flight collections [15,16]. An aerobiology sampling mission at 20 km elevation recovered two fungi, *Penicillium* and *Eurotiomycetes*, and *Bacillus subtilis* and *B. endophyticus*, as well as a very slow-growing *Bacillus* strain, which likely originated from trans-Pacific dust (Table 1) [17]. Most recently, a microbial aerosol sampling mission cultured *Bacillus*, *Paenibacillus*, *Actinobacteria* and *Proteobacteria* [10], (NC Bryan *et al.*, abstract 7523, Astrobiology Science Conference, Chicago, IL, 2015). These investigators also estimated metabolic activity from ATP measurements in biomass and suggested densities of 10^5–10^6 microbial cells/m^3 in the stratosphere.

While recent studies have clearly established that viable and diverse microorganisms are present in the stratosphere (Table 1), largely dispelling earlier sterility and

Table 1

Microorganisms isolated or tested in the stratosphere

Microorganisms	Collected (km)	Tested (km)	References and notes
Archaea			
Halobacterium sp. NRC-1		36	[4••]
Halorubrum lacusprofundi		36	[4••]
Bacteria			
Actinobacteria	18–29		[a]
Bacillus endophyticus	20		[17]
Bacillus luciferensis	30		[13]
Bacillus pulmilus SAFR-032		20	[26••]
Bacillus simplex	41		[12]
Bacillus sphaericus	20		[13]
Bacillus spp.	11–77		[16,17] [a,b,c,d,g]
Bacillus subtilis NASA8	20	20	[17,1] [g]
Brevibacterium luteolum	20		[13]
Deinococcus aetherius ST0316[T]	10–12		[15] [g]
Deinococcus sp. TR0125	10–12		[15]
Escherichia coli		40	[5••]
Microbacteriaceae	20		[13]
Micrococcus sp.	20		[14]
Micrococcus albus	48–77		[c,d,g]
Mycobacterium luteum	48–77		[c,d,g]
Paenibacillus sp.	12–35		[16] [g,a]
Proteobacteria	18–29		[a]
Proteus mirabilis		40	[5••]
Pseudomonas aeruginosa		40	[5••]
Salmonella typhimurium		40	[5••]
Staphylococcus sp.	20		[13]
Staphylococcus aureus (MRSA)		40	[5••]
Staphylococcus aureus		40	[5••]
Staphylococcus pasteuri	41		[12]
Fungi			
Actinomyces sp.		19	[e]
Aspergillus fumigates	11–21		[b]
Aspergillus niger	11–21, 48–77	19, 22	[b,c,d,e,g]
Brachysporium sp.		22	[e]
Circinella muscae	48–77		[c,d,g]
Cladosporium sp.		22	[e]
Diplodia sp.		22	[e]
Engyodontium albus	41		[12]
Eurotiomycetes sp.	20		[17]
Fusarium sp.		19	[e]
Helminthosporium sativum		22	[e]
Hysterium sp.		22	[e]
Macrosporium sp.		19	[e]
Macrosporium	11–21		[b]
Monilia sitophila		19	[e]
Papulaspora anomala	48–77		[c]
Penicillium cyclopium	11–21		[b]
Penicillium notatum	48–77		[c,d,g]
Penicillium sp.	20, 40	19	[13,17] [e,f]
Pestalozzia sp.		19	[e]
Puccinia graminis		19	[e]
Rhizopus sp.	11–21	19, 22	[b]

[a] NC Bryan *et al*., abstract 7523, Astrobiology Science Conference, Chicago, IL, 2015.
[b] LA Rogers, FC Meier US Army Air Corps Stratosphere Flight of 1935 in Balloon Explorer II. National Geographic Society 1936:146–151.
[c] AA Imshenetsky *et al.*, Life sciences and space research XIV. Proceedings of the Open Meeting of the Working Group on Space Biology of the Eighteenth Plenary Meeting of COSPAR. 1976:359–362.
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[f] GA Soffen *Proceedings of the Atmospheric Biology Conference.* University of Minnesota Press; Minnesota 1965:213–219.
[g] Studies conducted on microbes post-collection.

contamination concerns, full description of the voluminous, but low density microbiome is still lacking. This is at least partly due to some remaining technical challenges: limited sampling by location, elevation, and duration [2], isolation of copious amounts of inorganic material along with organic matter, and low efficiency in culturing of viable microorganisms (~0.1 to 10% success) [10,11••,18•]. In addition, most efforts utilized media and culturing techniques optimized for mesophilic aerobic species and precluded growth of many extremophiles (e.g. halophiles). Modern non-culturing approaches for community analysis, for example, metagenomics and single cell genomics, have the potential to yield a much deeper understanding of the stratospheric microbiome; however, to date, few such atmospheric studies have been conducted, with none so far in the stratosphere [7•,19–22,23•,24••].

Mechanisms of microbial survival in stratospheric conditions

Recent studies have attempted to determine relevant extremophilic properties of microbial isolates after subjecting them to stratospheric stressors through flight exposures and simulations in the laboratory (Box 1). These studies have exposed microbes to a smorgasbord of challenging conditions, high doses of UV-C radiation causing DNA lesions, hypobaric and desiccating conditions resulting in double-stranded DNA breaks, freezing temperatures retarding growth and metabolic activities, and other stressors. An important factor, atmospheric residency time, estimated to be from days to years, depending on weather events such as wind speed and direction, particle size, humidity levels, and temperature, has been largely unexplored [21,24••].

In a comparative study, monolayers of endospores from *B. subtilis* NASA8 (isolated from 20 km) and a desert isolate (WN696) were exposed to stratospheric conditions (20 km for up to six hours) or simulated conditions [1]. Combinations of UV irradiation, low temperature, desiccation, and reduced pressure were tested, with or without a simulated atmospheric dust coating made from volcanic field material. The results confirmed findings from an earlier ground simulation experiment using *B. subtilis* (HA 101) endospores [25]. Significant loss of viability was found both in the stratosphere and in laboratory studies, with the primary cause being cumulative UV exposure, resulting in <0.1% viability after six hours. The study suggested that microniches in surfaces and dust particles may shelter cells from harmful radiation and enhance survival [1]. The study surprisingly also concluded that exposure to additional environmental stressors did not enhance lethality.

In a similar study, monolayers of *Bacillus pumilus* endospores from a spacecraft cleanroom were spotted onto metal surfaces and either exposed to sunlight or shielded in the stratosphere at 31 km or subjected to UV-C in the laboratory [26••]. Spores exposed to stratospheric sunlight showed progressive decline with increased exposure time, and the phenomenon was reproducible in the laboratory. These investigators found that stratospheric lethality resulted primarily from UV exposure, with only 0.001% viability after eight hours of sunlight exposure in the stratosphere. However, when the endospores were stacked rather than in monolayers in the laboratory, the UV-C effects appeared to be lost by shielding. This study expanded a previous investigation of *B. subtilis* endospores [25]. Surprisingly, genome sequencing of the *B. pumilus* strains revealed only three single nucleotide differences between stratosphere and ground cultures [26••]. Sequencing of *B. pumilus* also revealed novel DNA repair genes which may potentially explain its exceptional stratospheric survival characteristics [27].

In two studies, UV resistance and other stress responses of *Deinococcus aetherius*, and endospores from *Bacillus* and *Paenibacillus* isolated from the stratosphere, were found to be similar to those of terrestrial isolates. In addition, *D. aetherius* exhibited high tolerance to desiccation and γ-radiation similar to *D. radiodurans*, and was found to aggregate in culture, which suggested this as a mechanism for shielding cells against deleterious radiation [15,16].

In a study of medically relevant laboratory and clinical strains, including methicillin-resistant *Staphylococcus aureus* (Table 1), lyophilized cells were exposed to stratospheric conditions on balloons launched to 40 km elevation and laboratory simulations [5••]. All flown samples showed growth inhibition over and above that observed for similar UV-C doses in the laboratory, highlighting the importance of the other stratospheric stressors. Interestingly, the study also found that protein expression was down-regulated, and metabolic pathways and antibiotic resistance patterns were altered after UV radiation.

In a study of two halophilic archaea, *Halobacterium* sp. NRC-1, a mesophile, and *Halorubrum lacusprofundi*, a biofilm-former from Antarctica, were launched in balloons to 36 km, with both withstanding the journey [4••]. The cold-adapted *H. lacusprofundi* exhibited superior survival which was correlated to better freeze-thaw tolerance in the laboratory. Genomic analysis showed extra copies of cold-shock genes and transcriptomic analysis indicated a more robust cold shock response in *H. lacusprofundi*. *Halobacterium* sp. NRC-1 displayed higher radiation tolerance, and its responses to UV-C and ionizing radiation were characterized by transcriptomic and survival experiments in the laboratory [28,29].

Microorganisms use varied strategies for maintaining viability in the extremely stressful stratosphere. Gram-

positive bacteria and fungi likely use sporulation as an important common survival mechanism (Table 1) [13], although where the spores form is not yet a resolved question. This is not surprising since endospores are known to survive lengthy periods in a cryptobiotic state. Non-spore-forming bacteria and certain archaea, in contrast, often possess G+C-rich genomes, which may increase UV tolerance and survival (Table 1) [30]. Several studies have confirmed an important role for UV radiation in loss of viability and shielding or reflective properties of dust to be protective. Clumping or biofilm-formation may protect the interior of a microbial community, while potentially sacrificing cells in outer layers. Additionally, efficient DNA repair systems, photoprotective pigments (e.g. carotenoids and melanins) and cold shock proteins have also been reported to play important roles in stratospheric survival [1]. While a few genomic and proteomic studies have provided clues on the mechanisms of survival in the stratosphere [4••,27,31••,32••,33•], many more are clearly needed.

Perspectives and implications: microorganisms in the stratosphere

Survival of microorganisms in the stratosphere may have considerable consequences for public health and agriculture. Airborne microbial cells circulating at measurable densities (~10^4 cells/m^3 [7•,10]) in the atmosphere likely result in the spread of infectious diseases and allergens. Some isolated strains from the stratosphere are pathogenic to plants and animals, and clinical isolates have been shown to survive at these high elevations [5••,20,34]. The potential public health and medical implications underscore the need for more detailed surveys of microbial transport through the atmosphere and investigations into the mechanisms of their survival [23•,24••,33•,35–37]. Such an endeavor should address the current lack of conformity of methods and materials which are needed to reach the ultimate goal of creating an atlas of the stratospheric microbiome.

Recent trends in global warming and climate change, such as accelerated desertification and escalating tropical storms, will likely intensify stratosphere-troposphere exchange, and enhance long-range dispersal of microorganisms [6,38••,39•]. Increased transfer of water vapor and particulate matter into the stratosphere may also improve habitability of resident microorganisms and adversely impact atmospheric ozone concentrations [40•,41]. Microorganisms in the troposphere are believed to affect climate, for example, by promoting ice nucleation, and their increased presence and actions in the stratosphere may also result in changes in precipitation. Some reports have even raised the possibility of their use in modulating climate [7•,24••,42•,43•,44]. Given the wide-ranging environmental concerns of climate change, additional investigations into the importance of microorganisms in the stratosphere are clearly warranted.

Stratospheric microbiology studies are helping refine planetary protection goals, both for missions to other bodies in the solar system and for return of samples back to Earth (e.g. [19]). These are helping to shed light on the potential bioburdens and effects of exposure on microbes needed to determine viability of hitchhikers on our transportation devices. The standards have evolved since the Viking Lander mission attempted to be fully sterile, with some scientists now claiming that this may be a near-impossible task and prohibitively expensive [45], while others arguing that the goal and its costs may be justified [25,46,47]. The prospect of a renewed space race and commercial space travel underscores the need for greater international discussion and coordination. Better understanding of microbial survival in stratospheric conditions is an important goal that will aid in improving the guidelines for planetary protection.

Finally, relevant to the field of astrobiology, expanding studies in stratospheric microbiology promotes our understanding of microbial survival of extreme conditions on Earth and beyond. For example, a number of stratospheric stressors such as low temperature, hypobaric pressure, desiccation, UV and cosmic radiation mirror those in space and on the surface of Mars [6,48]. How combinations of various stressors affect health and survival of cells is an important area for future investigation and raises the potential for practical applications of polyextremophiles [49]. Stratospheric microbiology studies may help ensure success in future space flights, including transferring and sustaining human life away from planet Earth. Such studies may also be relevant to assessing the potential for life on the many exoplanets which have been discovered [50].

Conclusions

Microbial survival requires extremophilic characteristics including tolerance to intense UV radiation, low pressure, lack of water and nutrients, and freezing temperatures. The stratosphere is likely the upper limit for life, combining some of the most challenging conditions found anywhere in our biosphere. Nevertheless, underscoring the heartiness of terrestrial microorganisms, numerous bacteria and fungi have been isolated and can survive at these elevations. The stressors may be overcome by sporulation and dormancy, aided by aggregation and shielding, as suggested for many of the viable stratospheric isolates. The ozone layer located largely within the stratosphere acts as a protective radiation shield for our planet, allowing terrestrial life forms to be transported and returned in a viable state from the troposphere and lower stratosphere. It is also clear that stratospheric microbiology raises a wide range of environmental concerns, from health and agriculture, and climate change, to

planetary protection and astrobiology, for which more comprehensive studies are certainly warranted in the future.

Conflict of interest

None declared.

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